Louisiana State University
LSU Digital Commons

LSU Historical Dissertations and Theses | Graduate School

1963

The Characterization of a Growth Inhibitor of Glandless Cottonseed.

Charles Johnston
Louisiana State University and Agricultural & Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_disstheses

Recommended Citation
Johnston, Charles, "The Characterization of a Growth Inhibitor of Glandless Cottonseed." (1963). *LSU Historical Dissertations and Theses*. 893.
https://digitalcommons.lsu.edu/gradschool_disstheses/893

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Historical Dissertations and Theses by an authorized administrator of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

This dissertation has been 64-5053
microfilmed exactly as received

JOHNSTON, Charles, 1937-
THE CHARACTERIZATION OF A GROWTH INHIBITOR OF GLANDLESS COTTONSEED.

Louisiana State University, Ph.D., 1963
Agriculture, animal culture

University Microfilms, Inc., Ann Arbor, Michigan

THE CHARACTERIZATION OF A GROWTH INHIBITOR OF GLANDLESS COTTONSEED

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Poultry Science

by
Charles Johnston
B.S., University of Arkansas, 1960
M.S., Louisiana State University, 1961
August, 1963

ACKNOWLEDGEMENT

The author wishes to convey his sincere appreciation to the following people for their aid given during the course of this study: to his wife for her encouragement and understanding, to Dr. A. B. Watts for his guidance and unending patience, to Mr. Garlon Harper of the National Cottonseed Products Association for his aid in obtaining the cottonseed and cottonseed meals, to Mr. Ernest Epps and Mr. Hugh Austin of the Feeds and Fertilizer Laboratory for the generosity with which they gave of their time and equipment, to Dr. W. W. Cravens of the McMillen Feed Division of Central Soya for his aid in obtaining the samples of soybean oil meal. Also, he wishes to thank the faculty and staff for their aid in collecting these data.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The beneficial value of extracting glandless cottonseed meats with an homogeneous hexane-acetone-water solvent system or mildly heating the meats and extracting the soluble material with hexane has been investigated. The meals were evaluated by two or three week growth trials, by nitrogen utilization studies and by infrared absorption spectral changes. In the growth trials, duplicate lots of chicks were fed a semi-purified ration in which the cottonseed meal supplied at least half of the total protein. In a growth trial designed to determine the effect of autoclaving on the glandless meals, nitrogen balances were determined for groups of four birds which had been fed the meals for three weeks.

Results of the growth trials are expressed as gain per chick day, as protein consumed per chick day and as protein efficiency ratios (gain per gram of protein consumed). Nitrogen utilization is expressed as the percentage of the nitrogen consumed which is utilized.

Recombination of the extracts with the meals from which they were removed or with soybean oil meal showed no difference in nutritional value of the unheated extracts.

Heating all extracts, except that from unheated hexane extracted glandless cottonseed, to 225° F. in thirty minutes significantly reduced their nutritional value. Corn oil gave similar results. This investigation shows that extraction of the glandless meats with hexane or the h.a.w. solvent mixture does not remove materials which are any more deleterious to the chick than those contained in corn oil.

The improved growth of the chicks fed the heated hexane extracted meal could be reversed by grinding the meal to a very fine particle size and vacuum drying the meal. These results plus the additional growth produced when the meal was first extracted with hexane without heat and then extracted with the hexane-acetone-water solvent strongly suggest that the observed effect is due to a physical and/or chemical change in the complex structure of the meal which requires the presence of water and heat or water and acetone before the change can take place. This observation was further substantiated when a meal which was produced by heating the meats to 180° F. in ten minutes, spraying the meats with 12 percent boiling water

and heating the meats for an additional 20 minutes promoted greater growth than any other cottonseed meal and was equal to soybean oil meal.

Attempts to increase the nutritional value of the unheated hexane extracted meal by autoclaving proved this heat treatment to be much too severe. As the time of autoclaving was increased up to 20 minutes with this meal, its nutritional value was decreased but its nitrogen utilization was increased 29 percent. Autoclaving the heated hexane extracted meal did not appreciably change its nutritional value or nitrogen utilization. The heated and unheated hexane-acetone-water extracted meals were reduced in nutritional value as the time of autoclaving was increased but their nitrogen utilization was unchanged.

Infrared spectra of the heated hexane extracted meal, the unheated hexane extracted meal and its fractions which were autoclaved for varying lengths of time and the unheated and heated hexane-acetone-water extracted meals reveal that the ---N ---H stretching wavelength (2.75 to 3.25 microns) and ---C = O stretching wavelength (6.05 microns) are altered with heat and the hexane-acetone-water solvent system. Changes at the --- N --- H stretching wavelength

appear to be associated with nutritional changes of greater magnitude and those at the C = O stretching wavelength with differences of lesser magnitude.

These results strongly suggest that the improvement in nutritive value of the unheated hexane extracted glandless cottonseed meal is due to changes which involve hydrogen bonding.

INTRODUCTION

The beneficial nutritional effects observed due to the heating of cottonseed during or after the extraction of the oil have been known for some time. However, it has been assumed that the heat effect was due entirely to the binding of the gossypol and gossypol like pigments to the other materials thereby making the toxic material unavailable to the animal.

Recent workers have hypothesized that gossypol like materials are not the only growth limiting qualities of glanded cottonseed but much of their work has been masked by the presence of gossypol and there was therefore no clear cut evidence to support this hypothesis. With the introduction of cottonseed meals prepared from cottonseed which had been bred to contain very few pigment glands in the seed it has been clearly demonstrated that gossypol is not the only growth limiting quality of cottonseed. Very exacting conditions of heating the seed prior to extraction or the type of solvent used in removing the oil must be met before an appreciable beneficial effect in the meals can be observed. When these exacting conditions of heat treatment or solvent system are met, the cottonseed meals produced from glandless

cottonseed are of high nutritive value.

It was the purpose of this study to determine if a deleterious component of the glandless cottonseed was being removed or destroyed by heat or the solvent system or if the change which takes place is due to a physical change in the complex structural components. After characterization of the effect it was also planned to chemically or physically determine and to measure the observed changes.

REVIEW OF LITERATURE

It was very early recognized that cottonseed meal could be converted to a suitable source of protein by the simple expedient of heat (26). The same effect was observed with extraction of the seed with diethyl ether prior to manufacture of the meal. This observation and related observations led to the "bound" gossypol theory of gossypol detoxification (3, 4, 5, 34). According to this theory, gossypol was assumed to be solely responsible for the toxicity of raw cottonseed and it was assumed, further, that the gossypol became inactivated during cooking of the cottonseed meal as a result of its reaction with the protein in the seed to form "bound" gossypol. Because of the bound gossypol theory and because a high correlation was found (34) between the decrease in the amount of gossypol which could be extracted with diethyl ether and the improved nutritive value of the cotton-seed meal gossypol was believed to be the only deleterious factor affecting nutritive value of glanded cottonseed meal. Since these early observations a great deal of research has been conducted to investigate methods of lowering the gossypol content of cottonseed meals or of removing the gossypol by extraction with various solvent systems so that the resulting

meal would be of high nutritive value when fed to monogastric animals as well as ruminant animals.

Heating the seed in a dry condition effected a change in the form of the gossypol but only slightly reduced its toxicity (9). Autoclaving the seed at twenty pounds pressure for one hour destroyed the gossypol and produced a non-toxic product. Chick tests with meals of initially high quality showed a progressive decrease in weight gain per gram of nitrogen as the time of autoclaving the meals increased (6). This indicated that one cause of variation in nutritional quality of cottonseed meals, provided the free gossypol was 0.04 percent or less, was the amount of heat to which they were subjected. The reduction in the protein index of the meals with increased time of autoclaving was paralleled by a similar decrease in the nitrogen solubility of the meal in 0.02 N sodium hydroxide. Similar results (25) were found with rat feeding experiments.

Chick growth rate was much less on hydraulic press meals than solvent extracted meals (28). Guinea pigs grew very well when fed cottonseed meals which were processed by the hydraulic press method (115 degrees centigrade for 90 minutes) with enough water to bring the total moisture content to 14.5 percent (17).

Marked variation in individual amino acid availability has been shown to be common among commercial hydraulic press cottonseed meals. Lysine and methionine were particularly low, with values of 64 and 67 percent, respectively. Lysine availability values as high as 85 percent were obtained with a special solvent and gland-free meals. Heat treatment as severe as autoclaving for one hour at fifteen pounds pressure did not reduce the availability of the essential amino acids in cottonseed meals of low oil content. Lysine was more sensitive to heat destruction than the other essential amino acids. About a ten percent reduction in lysine was observed when solvent processed cottonseed meal was autoclaved at fifteen pounds pressure for sixty minutes (15). A two hour autoclaving period reduced the nutritive value of cottonseed meal approximately seventy percent (19) and reduced the lysine content of cottonseed thirty-five percent (7). These results also indicated that the simple measurement of the solubility of the nitrogen in the meals, commonly used to indicate the degree of heat damage, does not reflect the complete change induced in cottonseed by heat. In further studies it was shown that one-fifth of the lysine content of the seed may be destroyed in conventional commercial operations, depending not only on the process used, but upon the severity of the process (19).

Other workers (20) assessed the relative importance of gossypol and raffinose in binding and destroying lysine and in impairing the nutritive value of cottonseed meal using protein depleted rats as the test animals. They concluded that raffinose in cottonseed meal reduced the lysine content and nutritive value of the protein when heat was applied. A one percent concentration of gossypol was not as effective as a ten percent concentration of raffinose in destroying lysine in the cottonseed. Gossypol and raffinose at the same concentration are comparable in reducing the level of free epsilon-amino lysine in cottonseed. The nutritive index of cottonseed meals as determined by the rat repletion method is highly correlated with the free epsilon-amino groups of lysine of the protein and poorly correlated with the total lysine. The reduction in nutritive value of processed cottonseed is therefore due not only to the lysine destruction but also to lysine binding. To obtain a cottonseed meal which would be of optimum nutritive value, having a high content of available lysine as well as having a low gossypol content, a new solvent system was introduced (14) composed of acetone-hexane-water in the proportions of 54, 44, and 3 percent, respectively. The cottonseed meals produced with this solvent system contained much less gossypol and much more available lysine than

commercially prepared glanded meals. Chick growth demonstrated that the meals were of superior nutritive value to other glanded cottonseed meals (18).

It is concluded that the "bound" gossypol theory is no longer the only explanation of the changes which occur when glanded cottonseed are heated. However, this appears to be the only beneficial effect observed. The heat treatment employed must be mild enough so that the lysine is not bound to raffinose or other components of the seed thereby leaving it available to the animal. At the same time enough heat must be applied to glanded seed to eliminate the toxic qualities of the gossypol (2, 11, 24, 26).

Hopi cotton was first reported by Lewton (16) and later by Fulton (8) to have a variable number of pigment glands in the boll. McMichael (22) in investigating the possibilities of crossing Hopi cotton with commercial upland varieties found by selecting for the number of pigment glands in the seed the gossypol content could be reduced nearly to zero. He found that the glandless characteristic could be transferred to upland strains after two or more generations of selection. The total gossypol was reduced from 1.31 percent in the normal seed to 0.22 percent in the glandless seed. Other workers confirmed this analysis (30) concluding that glandless cottonseed

is essentially free of gossypol, giving very low "free" gossypol values as compared to gland containing cottonseed (21). Their analyses of glandless seed yielded free gossypol values of about 0.002 percent and total gossypol values of about 0.40 percent. The oil removed from the two types of seed was similar. Location in which the cotton was grown did not have any effect of the nutritive values of the cottonseed meals produced as shown in rat growth studies (30) and the glandless cottonseed meals were shown to be of superior nutritive value to cottonseed meals of the glanded type.

In chick growth studies (13) it was found that when the meals were prepared by extracting the seed with commercial hexane and air dried they were of significantly lower nutritive value than the same meals when they were mildly heated after flaking and steam dried. For purposes other than binding the gossypol this was the first time that heat treatment had been shown to be clearly beneficial to cottonseed. When the glandless meals were prepared by extracting the glandless seed with the hexane-acetone-water solvent system, which had been shown previously to produce high quality glanded cottonseed meal, the resulting meals were of equal nutritive value to those produced by extracting the glandless seed with hexane with the addition of heat. The addition of mild heat prior to extraction with

the solvent mixture did not exert a beneficial effect and a slight decrease in the nutritive value of the glandless cottonseed meal was observed. These data suggested that the hexane-acetone-water solvent mixture removed or destroyed a growth depressing factor or factors which were also removed or destroyed when the seed has been heated and extracted with hexane, thereby permitting the glandless cottonseed to be improved significantly in nutritional value.

MATERIALS AND METHODS

All chicks used in this study were straight run commercial broiler type which were purchased from a commercial hatchery. All chicks were housed at one day of age in electrically heated battery type brooders with raised wire floors. Each deck of the five deck batteries was divided with a Masonite sheet and one replication of ten chicks placed in each side of the deck. The chicks remained in the divided deck pens until the treatment was terminated or until they were four weeks of age, whichever came first. As the criterion of growth, protein efficiency ratios (grams gain per gram of protein consumed), protein consumed per chick day, and gain per chick day were used. All ingredients used in the semi-purified type rations, except the cottonseed meal under investigation, were of commercial origin and had proven to be of consistently high quality.

Nine hundred pounds of glandless cottonseed which had had all of the fiber except the "fuzzy" lint removed were obtained from the Shafter Experiment Station, Shafter, California. All cottonseed meals prepared by Louisiana State University came from this shipment of seed. On arrival, the seed were removed from the sacks and stored in metal containers

at room temperature until being processed.

The seed was prepared for extration of the oil in the following manner. The hulls were cracked and the meats liberated by passing the seed through a burr mill at a setting so that cracking occurred with very little pulverizing. Additional meat separation was accomplished by shaking the material through a series of screens attached to a mechanical shaker. The screens used were four, eight and sixteen mesh, respectively. Three passes through the four and eight mesh screens and five passes through the sixteen mesh screens were required to remove the maximum amount of extraneous material from the meats. After each screening, the lint materials were removed from the top of the meats and discarded while the meats remaining on the screen were deposited in a container until the screening process was complete at which time all the meats were mixed together. The almost lint free meats were then ground through a Wiley Mill with one-eighth inch screen to simulate the rolling and flaking process used to prepare previous meals (13). The meats were then considered to be ready for the oil to be extracted and the meals to be prepared.

All extractions were carried out in two, three, or five gallon wide mouth glass jars. The size container used being determined by the volume required to contain the meats and

enough solvent so that the solvent to meats ratio was two and one-half to one with enough space remaining to facilitate proper shaking.

After the initial solvent soaking period of thirty minutes, and after each successive soaking period of from one to six hours the miscella and what fine materials which would not settle out were siphoned off and fresh solvent added. Twelve solvent passes were made in the extraction of the oil from each batch of seed. Fresh solvent was used with 6 of the 12 extractions with the mixed solvent system. The original thirty-three and a half percent hexane, sixty-four and a half percent acetone and two percent (volume for volume) water ratio was restored by adding water to the recovered solvent until two phases separated and then adding acetone until the two phases disappeared. The reclaimed hexane from those meats which were extracted only with hexane was used repeatedly.

After the miscella was siphoned off the meats, the fine particles of cottonseed, which would not settle, were removed by filtering the miscella through a Buchner funnel under reduced pressure with coarse filter paper (Whatman Number 1) and a layer of Hy-flo Super Cel. The resulting filtrate material contained no fine particles and was uniform in color.

The main portion of the solvent was removed from the

extract in a large volume extractor under reduced pressure in an atmosphere of dry nitrogen which was permitted to bubble through the material at all times. Heat was applied to the flask containing the solvent and extract until the major portion of the solvent was removed and the temperature began to rise in the flask. The heat was then removed and the remainder of the solvent removed under reduced pressure without heat. The extract was considered to be solvent free when the odor of hexane or acetone could no longer be detected. The extract was then stored in a freezer after being purged with dry nitrogen for two minutes.

All meals were desolventized after extraction by spreading on galvanized metal trays at a thickness of about one-half inch. The meals remained spread on the trays for twenty-four hours at which time only slight traces of the solvent could be detected. To facilitate complete removal of the solvent, the meals were dried for twenty-four hours in an unheated vacuum drying oven. After this drying procedure, the odor of the solvents could no longer be detected and only traces of moisture remained.

Except for Trial I all rations were formulated to be isocaloric and isonitrogenous within each trial by making one basal mix for each trial, adding the cottonseed meal

which was required in the greatest quantity and diluting the other meals with cellulose to provide the same quantity of protein to the total ration.

Large and small chicks were removed and the remainder allotted randomly to the various treatments.

The chicks were wing banded and individual weights recorded at the beginning of the experiment with the chicks which were on a preliminary depletion ration being weighed initially at seven days of age. Additional chick weights were recorded after an experimental duration of two weeks or when the experiment was terminated.

Two replications of ten chicks each were placed on each treatment in all experiments.

Light was supplied by fluorescent fixtures and was uniformly dispersed over all treatments.

Water and feed were supplied ad libitum with records being kept of the amount of feed consumed.

MATERIALS AND METHODS

EXPERIMENT NUMBER ONE

To isolate and characterize the effects of mild heat treatment or of extraction of the oil with the mixed solvent, it was necessary to reproduce the conditions by which these effects had been previously observed.

Two meals were prepared in the first experiment; the first was prepared by extracting the oil with the mixed solvent of hexane, acetone and water in the proportions mentioned previously and the second was prepared by extracting the oil with n-hexane. The two lots of meals were subdivided into nine equal sublots of sufficient quantity to furnish enough twenty-one percent protein ration to feed two replications of ten birds each for a period of two weeks.

Because the beneficial effect appeared when the meals were prepared by being extracted with the mixed solvent or when the seed were heated prior to extraction with hexane, three sublots of the seed were subjected to additional treatment after having been extracted with hexane. To one sublot twelve percent water was added to bring the total moisture content to 14.5 percent and the meal was heated in a convection type oven for a period of ten minutes at one hundred and eighty degrees F. and then

raised to two hundred and twenty-five degrees F. for a period of twenty minutes. Two other sublots were treated again by extracting with the mixed solvent twelve times. The various combinations of the meals, their treatment and the extracts added are shown on Table I. The extracts were added back to the meals on the basis of the amounts contained in the original meats. The rations were therefore isonitrogenous at a protein level of twenty-one percent but isocaloric only where each separate extract was added or where no extract was added. Seven percent corn oil was added to all rations and the total metabolizable energy content per pound of finished feed was approximately 926 Calories.

Typical extraction data are shown on Table II.

Chel 138 (ethylenediamine (di (o-hydroxyphenyl acetic acid) was included in fraction number 5 to determine under these conditions its ability to produce an increase in growth or feed efficiency.

A typical ration is shown on Table III.

Table I

Fraction Preparation for Experiment I

Fraction Number	Meal Preparation	Extract Added & Treatment	Protein Percent
1.	H.A.W. extracted	none	42.44
2.	H.A.W. extracted	H.A.W.	42.44
3.	H.A.W. extracted	H.A.W. heated	42.44
4.	H.A.W. extracted	hexane	42.44
5.	H.A.W. extracted plus 803 gm./ton of Chel 138	H.A.W.	42.44
6.	hexane extracted	none	43.25
7.	hexane extracted plus heat	none	43.25
8.	hexane extracted plus H.A.W. extracted	none	42.60
9.	hexane extracted plus H.A.W. extracted	H.A.W.	42.60

Table II

Extraction Data of Meal Preparation for Experiment I

H.A.W. Extraction		Hexane Extraction	
Meat weight before extraction*	25.5 lbs.	Meat weight before extraction	23.0 lbs.
Meal weight after extraction	18.5 lbs.	Meal weight after extraction	15.5 lbs.
Weight material removed or lost in extraction	7.0 lbs.	Weight material removed or lost in extraction	7.5 lbs.
Percent material removed or lost in extraction	27.4	Percent material removed or lost in extraction	32.6

*Meal and meat weights are on air dry basis.

Table III

Composition of a Typical Ration Fed in Experiment I

Material	Quantity (as per cent of ration)
Ground yellow corn	44.25
Cottonseed meal (42.44%)	39.25
Fermacto 400	1.25
Alfalfa leaf meal (17%)	3.00
Salt	0.50
Corn oil	7.00
Dicalcium phosphate	2.25
Oyster shell flour	1.50
Vitamin, mineral, antibiotic premix	1.00

The following quantities of materials supplied per pound of finished feed in the vitamin, mineral and antibiotic premix.

Material	Quantity
Vitamin A	2963 I.U.
Vitamin D_3	625 I.C.U.
Vitamin E	1.25 mg.
Vitamin K	0.28 mg.
Thiamine	0.97 mg.
Riboflavin	4.44 mg.
Calcium pantothenate	6.00 mg.
Niacin	14.50 mg.
Pyridoxine	1.60 mg.
Choline	731.60 mg.
Folacin	0.30 mg.
Vitamin B_{12}	0.004 mg.
Procaine penicillin	4.00 mg.
Copper	42 p.p.m.
Zinc	49 p.p.m.
Manganese sulfate (70%)	11.40 gms.
DPPD	1.00 mg.

RESULTS AND DISCUSSION

EXPERIMENT NUMBER ONE

The h.a.w. solvent mixture used in preparing these meals was of a different proportion than that proposed by Mann, et al. (18) because it was found by mixing in the reported proportions (44 percent hexane: 53 percent acetone: 3 percent water) a two phase system resulted and it is doubtful that any water was added to the meals until the solvent from the bottom of the container was added. By mixing in the proportions of 33.5 percent hexane: 64.5 percent acetone: 2 percent water an homogeneous solvent mixture was obtained.

The extraction data (Table II) for the two solvent systems shows that 5.4 percent more material was removed with hexane than was removed with the h.a.w. solvent. The larger figure for hexane extraction is due to the fine particles not settling as rapidly as the fine particles of the h.a.w. system. When the solvent and extract were siphoned from the meats, a larger part of the meal was also removed with the hexane. If the extract only is considered, about 2 percent more material is removed with the h.a.w. solvent system than with hexane.

Table IV

Results of Experiment Number One

Fraction and Ration Number	Gain per Chick day (gms.)	Protein Consumption per Chick day (gms.)	Protein Efficiency Ratio*
1.	8.28 ± .16	2.84 ± .04	2.94 ± .02
2.	5.58 ± .34	2.18 ± .09	2.56 ± .05
3.	5.29 ± .13	2.05 ± .02	2.59 ± .08
4.	5.19 ± .11	2.05 ± .10	2.54 ± .08
5.	5.00 ± .16	2.04 ± .00	2.45 ± .08
6.	7.27 ± .55	3.15 ± .07	2.31 ± .13
7.	7.59 ± .15	3.19 ± .02	2.39 ± .06
8.	8.03 ± .14	3.16 ± .01	2.54 ± .05
9.	8.45 ± .16	3.01 ± .005	2.81 ± .06

*Gain per gram of protein consumed.

The results of this experiment and the standard error of each mean are given in Table IV. The gain per chick day and protein consumed per chick day are included in addition to the protein efficiency ratios (grams gain per gram of protein consumed) because this figure is relative and does not reflect the absolute amounts.

The results show that extraction of the glandless meats with the mixed solvent (Fraction 1) produced a meal which the chicks consumed well and on which they also gained well. Utilization as determined by the protein efficiency ratio (p.e.r.) of this meal was the highest of all rations. When a proportionate amount of the extract was added back to the ration (Fraction 2) there was a significant decrease in gain and protein consumption. The protein which was consumed was not utilized as well as that of the preceding fraction giving a p.e.r. value of 2.56. A beneficial effect was not derived from heating the extract (Fraction 3) and there was a decrease in consumption as well as gain. However, the meal was utilized as well as the preceding preparation and yielded a p.e.r. value of 2.59. The addition of hexane extract to the h.a.w. extracted meal (Fraction 4) also resulted in a significant depression in growth as compared to the chicks which were fed the same meal to which no extract was added. Very similar

results were obtained by supplementing with this extract as were obtained with the addition of the heated h.a.w. extract.

The addition of Chel-138 at a level equivalent to 803 grams per ton to fraction 5 produced slightly less gain and therefore a lower protein efficiency than the other fractions which contained added h.a.w. extract. This observation is in accordance with the work of Miles (23) in which the same observation was made when the chelating agent was incorporated into practical type corn-soy rations. The mode of action of the depression is believed to be the binding of iron or copper in the intestine thereby making these elements unavailable to the animal.

Extraction of the meats with hexane (Fraction 6) produced meals on which the chicks gained more and consumed more protein but with a lower p.e.r. than the rations containing the h.a.w. prepared meals. The addition of heat (Fraction 7) to the hexane extracted meal increased the gain but the protein consumption was also increased proportionately. The p.e.r. therefore was about the same as that of the preceding fraction. Extracting with the h.a.w. solvent mixture after extracting with hexane (Fraction 8) significantly increased the gain. However, the protein consumption was also increased giving a p.e.r. value of the same magnitude

as the h.a.w. extracted meals to which the same extract was added. The addition of the small amount of h.a.w. extract removed after extraction with hexane (Fraction 9) attempted to produce a higher gain and protein consumption with a p.e.r. value equal to that obtained by extracting the meats with the mixed solvent alone. The tests with these last two fractions show that additional material is removed by extracting the meals with the mixed solvent system, and that the material removed is not deleterious to the nutritional value of the meal.

These results suggest that a deleterious component of the meats was removed by both of the solvent systems. An additional beneficial effect was observed when the meals were extracted with the mixed solvent after having been extracted with hexane but a reversal of this effect was not observed when the h.a.w. extract recombined at the same level at which it had been removed.

The conditions of heating the meals in this experiment did not produce the magnitude of change in the nutritive value of the meals which had been observed in previous work (13). This is believed to be due to an uneven dispersion of the added water in the meal and the uneven heat distribution because the meals were not agitated as heat was applied.

It is concluded from these results that two growth depressing factors may be present in glandless cottonseed. One factor which is present in the extracts and becomes more pronounced when the extracts are heated and one which is present in the meals. The data indicate that the factor in the meal is not removed by extraction but may be destroyed by extraction with the h.a.w. solvent mixture or with mild heat and water. It is indicated that the improvement in the nutritional value of the glandless meats when they are extracted with the h.a.w. solvent mixture or are heated with added water prior to extraction with hexane over hexane extraction alone is due to a physical or chemical change.

MATERIALS AND METHODS

EXPERIMENT NUMBER TWO

To independently examine the meals and extracts for the presence of deleterious growth materials and to further investigate the effect of different treatments on the meals and extracts were the purposes of this experiment. Meats prepared in the same manner as those in the previous experiment were used. Before extraction, the meats were divided into three equal lots, each of which was of sufficient quantity to prepare enough ration to feed four treatments with two replications of chicks for two weeks at a protein level of twelve percent.

Chicks used in this experiment were placed on a protein depletion ration for seven days to reduce the effect of the unabsorbed yolk and obviously weak chicks. Ground yellow corn was the only source of protein in the depletion ration and supplied eight percent. The composition of the depletion ration is shown on Table V.

To test the extracts, six percent protein from commercial soybean oil meal was used and the extracts were added at the five percent level. To test the cottonseed meals, which supplied six percent protein to the finished ration, corn

oil was added at the same level as the extracts. Yellow corn supplied six percent protein to all rations. By formulating the rations in this manner, all were isonitrogenous and isocaloric by weight of material added (approximately 1400M.E. Calories per pound of finished feed). The total protein content of each ration was twelve percent and the total extract of corn oil was five percent. The composition of a typical assay ration is shown in Table VI.

The mixed solvent extracted meal was subdivided into three sublots to determine the effect of grinding to an extremely fine particle size and the effect of additional vacuum drying after the meals were ground to an extremely fine particle size in a ball mill for twenty-four hours. Two extracts were tested from this extraction. One extract was heated to two hundred and twenty-five degrees F. for thirty minutes and the other was tested without heat treatment. If the extract contained a growth depressing material it should be destroyed by the heat treatment thereby yielding an increase in growth.

After being vacuum dried, the hexane extracted lot of meal was divided into four sublots. One sublot was untreated; to a second sublot twelve percent water was added and the meal was heated at one hundred and eighty degrees F. for ten

minutes and at two hundred and twenty-five degrees F. for twenty minutes. A third sublot of meal was ground in a ball mill for twenty-four hours and vacuum dried while a fourth sublot was adjusted to fourteen and a half percent moisture, heated at two hundred and twenty-five degrees F., and vacuum dried at one hundred and eighty degrees F. for twenty-four hours. Two samples of extract from this lot were tested, one of which was heated at two hundred and twenty-five degrees F. for thirty minutes and the other being untreated.

A third lot of meal was prepared for this experiment by heating the meats with twelve percent added water for ten minutes at one hundred and eighty degrees F. and at two hundred and twenty-five degrees F. for twenty minutes before the oil was extracted with hexane. This test should have more clearly indicated if there was a combination of two factors present or if one was involved. The meal prepared was subdivided into three sublots, two of which were subjected to additional treatment. The first sublot was ground in a ball mill for twenty-four hours and the second was vacuum dried for twenty-four hours at one hundred and eighty degrees F. Two extracts were tested from this extraction, one of which was untreated and the other was heated at an oven setting of two hundred and twenty-five degrees F. for twenty

minutes.

One additional lot of meal was prepared to determine the effect of the addition of boiling water to the unextracted meats which were heated for ten minutes at one hundred and eighty degrees F. before the water was added and heated an additional twenty minutes at two hundred and twenty-five degrees F. before being extracted with hexane and vacuum dried.

Two soybean oil meal control rations were included, one of which contained five percent heated corn oil (two hundred and twenty-five degrees F.) and the other which contained unheated corn oil.

The fraction preparation and the feeding regime are shown in Table VII, where fraction number and ration number are the same.

By preparing the meals and extracts in this way it was hoped to gain additional knowledge of whether the effect observed was due to a material being removed or destroyed or if the effect was due to a physical or chemical change in the protein of the seed by the mixed solvent or heat.

Table V

Composition of Protein Depletion Ration

Component	Percent of Ration
Ground yellow corn	94.25
Dicalcium phosphate	3.50
Ground oyster shell flour	0.75
Salt	0.50
Vitamin, mineral, antibiotic premix*	1.00

*Supplies the following per pound of finished feed.

Material	Quantity
Vitamin A	3405 I.U.
Vitamin D_3	341 I.C.U.
Vitamin E	2.5 mg.
Riboflavin	3.0 mg.
Calcium pantothenate	6.0 mg.
Niacin	14.0 mg.
Choline	720.0 mg.
Vitamin B_{12}	6.0 mcgm.
Procaine pencillin	4.0 mg.
Copper	42.0 p.p.m.
Zinc	49.0 p.p.m.

Table VI

Composition of a Typical Protein Assay Ration

Component	Percent of Ration
Ground yellow corn	71.00
Dicalcium phosphate	3.50
Oyster shell flour	0.75
Salt	0.50
Corn oil or extract	5.00
Cottonseed meal or soybean oil meal	14.10
Vitamin, mineral, antibiotic premix*	1.00
Ground rice hulls	4.15

*Same as in protein depletion ration.

Table VII

Fraction Preparation and Feeding Regime of

Materials Prepared in Experiment Number Two

Ration Number	Extraction Method	Meat Treatment	Meal Treatment	Percent Protein (Meal)	Oil or Extract	Heat Treatment
1.	h.a.w.	none	none	43.69	corn oil	none
2.	h.a.w.	none	vac. dried 24 hrs.	45.09	corn oil	none
3.	h.a.w.	none	ground in ball mill 24 hrs.	43.59	corn oil	none
4.	s.b.o.m.	----	----	47.59	h.a.w.	225° F. for 30 min.
5.	s.b.o.m.	----	----	ibid.	h.a.w.	none
6.	hexane	none	none	47.44	corn oil	none
7.	hexane	none	heated with 12% water for 30 min.	45.41	corn oil	none

Table VII (cont'd)

Ration Number	Extraction Method	Meat Treatment	Meal Treatment	Percent Protein (Meal)	Oil or Extract	Heat Treatment
8.	hexane	none	ground in ball mill 24 hrs.	48.15	corn oil	none
9.	hexane	none	heated with 12% water at 225° F. for 30 min. & vac. dried at 180° F. 24 hrs.	50.50	corn oil	none
10.	s.b.o.m.	----	----	47.59	hexane	225° F. for 30 min.
11.	s.b.o.m.	----	----	ibid.	hexane	none
12.	hexane	heated with 12% water at 225° F. for 30 min.	none	45.09	corn oil	none

Table VII (cont'd)

Ration Number	Extraction Method	Meat Treatment	Meal Treatment	Percent Protein (Meal)	Oil or Extract	Heat Treatment
13.	hexane	ibid.	ground in ball mill 24 hrs.	42.59	corn oil	none
14.	hexane	ibid.	ground in ball mill 24 hrs. & vac. dried 24 hrs.	44.30	corn oil	none
15.	s.b.o.m.	----	----	47.59	hexane from ext. no. 12	225° F. for 30 min.
16.	s.b.o.m.	----	----	ibid.	hexane from ext. no. 12	none
17.	hexane	heated at 180° F. for 10 min., 12% boiling water added & heated at 225° F. for 20 min.	none	46.19	corn oil	none

Table VII (cont'd)

Ration Number	Extraction Method	Meat Treatment	Meal Treatment	Percent Protein (Meal)	Oil or Extract	Heat Treatment
18.	s.b.o.m.	----	----	47.59	corn oil	225° F. for 30 min.
19.	s.b.o.m.	----	----	47.59	corn oil	none

RESULTS AND DISCUSSION

EXPERIMENT NUMBER TWO

The results of chick feeding evaluations of the meals and extracts are shown on Table VIII.

Vacuum drying or grinding to an extremely fine particle size did not affect the nutritional value of the h.a.w. prepared meals (Fractions 1-3). Heating of the h.a.w. extract (Fraction 4) did not change its nutritional value but the extract when tested with soybean oil meal was of significantly greater nutritive value (Fractions 4 and 5 vs. 1-3).

Heating the hexane extracted meal with 12 percent water for thirty minutes resulted in a significant gain and protein consumption but the protein efficiency ratios were the same (Fractions 6 vs. 7). Grinding the unheated meal to a finer particle size did not significantly effect its nutritional value (Fraction 6 vs. 8). Heating the meal with 12 percent water and vacuum drying the meal for 24 hours at 180° F. significantly lowered the gain and protein consumption but there was not a significant lowering of the protein efficiency (Fraction 6 vs. 9). Heating the extract from the unheated hexane extracted meal resulted in a significant improvement in gain but not in protein consumption or the protein efficiency

(Fraction 10 vs. 11).

The meals prepared by heating the meats with 12 percent water before extraction yielded significantly lower gains, protein consumption and protein efficiencies when it was ground in a ball mill to a fine particle size (Fraction 12 vs. 13 and 14). This deleterious effect was emphasized significantly when the finely ground meal was vacuum dried for 24 hours (Fraction 13 vs. 14). Heating the hexane extract from these meals decreased the gain and protein consumption approximately 50 percent and significantly lowered its protein efficiency (Fraction 15 vs. 16).

Heating the meats to 180° F. for ten minutes and adjusting the moisture content to 14.5 percent with boiling water before extraction with hexane (Fraction 17) produced the greatest gain and protein consumption of all cottonseed meal rations and equal to the control ration of soybean oil meal with unheated corn oil.

The nutritional value of corn oil was reduced over 50 percent when it was heated under the same conditions as the extracts of the previous meals (Fraction 18 vs. 19).

Extraction with hexane produced meals in this trial which were of equal or greater nutritive value to those produced by extracting the oil with the h.a.w. solvent system.

Table VIII

Results of Experiment Number Two

Ration and Fraction Number	Gain/ Chick day (gms.)	Protein Consumed/ Chick day (gms.)	Protein Efficiency Ration*
1.	3.10 ± .35	1.24 ± .077	2.48 ± .010
2.	3.30 ± .10	1.29 ± .055	2.52 ± .160
3.	3.10 ± .20	1.21 ± .086	**2.56** ± .010
4.	6.20 ± .10	1.81 ± .095	3.43 ± .130
5.	6.50 ± .40	1.91 ± .150	3.42 ± .060
6.	3.40 ± .10	1.32 ± .030	2.60 ± .135
7.	3.70 ± .15	1.43 ± .010	2.56 ± .120
8.	3.00 ± .25	1.19 ± .095	2.48 ± .010
9.	2.90 ± .10	1.21 ± .015	2.41 ± .050
10.	6.60 ± .20	1.96 ± .045	3.37 ± .040
11.	6.10 ± .10	1.90 ± .035	3.22 ± .110
12.	5.35 ± .25	1.61 ± .041	3.27 ± .080
13.	3.50 ± .44	1.36 ± .165	2.54 ± .350
14.	2.30 ± .25	1.03 ± .050	2.17 ± .140
15.	2.40 ± .10	1.09 ± .030	2.22 ± .155
16.	6.90 ± .15	2.03 ± .065	3.39 ± .015
17.	6.60 ± .25	1.93 ± .165	3.40 ± .150
18.	3.00 ± .10	1.25 ± .005	2.42 ± .035
19.	6.40 ± .20	1.91 ± .010	3.37 ± .055

*Gms. gain per gram of protein consumed.

Extraction with hexane after 12 percent water had been added to the meats produced meal which was of significantly greater nutritional value than the h.a.w. extracted meal and the meal which was prepared by extracting the meats with hexane without the additions of water or mild heat (Fraction 12 vs. 1 and 6).

Vacuum drying exerted a detrimental effect where additional water was added back to the meal after initial vacuum drying or where the meals were vacuum dried after being ground to a very fine particle size (Fraction 6 vs. 7 and 8). Therefore, under these conditions, it is hypothesized that the effect is due to a reversal of the beneficial effects of heat which is perhaps a renaturation process.

The beneficial effect on gain obtained by heating the extract from the meal to which no water had been added (Fraction 10 vs. 11) suggests the presence of a growth inhibitor which is destroyed by heat and permits equal growth to that obtained by the other unheated extracts. All other heated extracts produced decreases in the amount of gain they would produce and the protein the chicks would consume in the presence of the heated extract. The reason for this depression produced by heating all of the other extracts including corn oil is not known. The most logical answer to this question is the formation free radicals from the

autoxidation of the oils which make the ration much less palatable to the chick and leads to lower feed consumption.

The failure of the h.a.w. prepared meals to be of as high nutritive value as those prepared in the previous experiment and those in which the original effect was observed (13) can be explained only on the basis that a more thorough extraction of the soluble materials from the other meals was accomplished in this experiment, or that all of the solvent was not removed from the meal by vacuum drying.

Preparation of glandless cottonseed meals by adding boiling water to the meats which are at 180° F. (Fraction 17) appears to be the method of choice. It is hypothesized that the reason for the high nutritive value of this meal over the others is that the addition of boiling water at this stage of extraction enables the heat applied during the other twenty minutes of heating to better destroy the toxic principle or enables a more desirable state of denaturation or chemical change to take place.

This investigation shows that extraction of the glandless meats with hexane or the h.a.w. solvent mixture does not remove materials which are any more deleterious to the chick than those contained in corn oil. When corn oil and the extracts from glandless cottonseed are heated to 225° F.

for thirty minutes a reduction in their nutritive value results. The reduction is much more dramatic with corn oil than for the extracts from glandless cottonseed.

The deleterious component of glandless cottonseed is therefore completely segregated into the meals where it must be destroyed or depressed in action by mild heat treatment in the presence of water or the proper type of solvent system. These results strongly suggest that the mild heat treatment or h.a.w. solvent system are exerting the same type of action which is most likely denaturation of the protein of the seed.

MATERIALS AND METHODS

EXPERIMENT NUMBER THREE

Since no detrimental effects were obtained by feeding the unheated extracts prepared in the two previous experiments, it was considered probable that the effects which were being observed were due to a physical or chemical change taking place in the meats when they were either heated or extracted with the h.a.w. solvent system. The investigation of this hypothesis was the purpose of this experiment.

The glandless cottonseed meals used in this experiment were obtained from the Southern Regional Laboratory of New Orleans where the h.a.w. extracted meals were prepared and the hexane extracted meals were stored. The hexane extracted meals were prepared completely at the Texas Engineering Experiment Station, College Station, Texas and sent to the Southern Regional Laboratory. These samples were from the same glandless meal lots in which the original heat and solvent effects were observed (13).

The hexane extracted heated meal was prepared in the following manner (33). The cottonseed, after having been delinted to three percent linters, were sprayed with enough water to give nine percent moisture in the meats after

standing for forty-eight hours in a closed container. After the forty-eight hour storage period the moisture content of the seed was eleven percent.

The seed were hulled and the meats separated with a Bauer Brothers separator. The meats were then immediately flaked on a Flak-All machine to a thickness of seventeen thousandths of an inch.

The heating which followed immediately after flaking was done in a twenty-two inch diameter French Oil Mill Machinery Company Cooker which was made of cast iron. The flat bottom was machined so that the agitator fitted close and kept the surface clean. The agitator also kept the meats turning and mixing during the cooking period. Both the bottom and side walls of the cooker were steam jacketed. Heat was applied at the proper rate by adjusting the steam pressure to heat the meats to one hundred and eighty degrees F. in ten minutes while being constantly stirred with the cover on the cooker. Moisture was added in the form of a water spray to bring the moisture content up to twelve percent and the cooking continued for an additional twenty minutes with the final temperature being two hundred and twenty-five degrees F. The cover was not removed from the cooker during the heating process.

After extraction with n-hexane, the flakes were desolventized in a continuous steam jacketed tube type of desolventizer which was equipped with a close fitting ribbon type conveyor agitator. Before desolventizing, five percent water was added to the extracted flakes. The residence time in the desolventizer was about twelve minutes. The temperature of the flakes leaving the desolventizer was two-hundred degrees F. and was no doubt somewhat higher inside the desolventizer.

The unheated hexane extracted meal was prepared in exactly the same manner as the heated except that prior to extraction of the oil there was no thirty minute heating period.

The heated and unheated h.a.w. extracted glandless meals were prepared at the Southern Regional Laboratory in the same manner described by Mann, et al. (18) for glanded meals.

The two methods of removing the oil differed materially in the type of solvent system used, the thickness at which the meats were flaked (0.003 inches for h.a.w. as compared to 0.017 for hexane) and the laboratory at which the extractions were made.

The solvent systems therefore were the major differences in the preparation of the meals with the heat treatment of

the h.a.w. extracted meals being as nearly the same as for the hexane extracted meals as was possible at the two different laboratories.

These conditions being so, it was decided to try to improve the nutritional value of the unheated hexane extracted meal by autoclaving the meal at 15 lbs. pressure for varying lengths of time.

In addition lots of the h.a.w. extracted heated and unheated meals were treated in the same manner as the two hexane extracted meals. Before autoclaving, the four meals were subdivided into four fractions of sufficient quantity to supply enough protein to feed two replications of ten chicks for a period of two weeks and to feed four birds from each treatment for an additional period of six days. The additional six day feeding period was included so that nitrogen balances could be determined on each sample.

For a comparison of the meals prepared at this station with those prepared elsewhere, the hexane unheated meal from the previous trial was included. One sublot of this meal was autoclaved at fifteen pounds pressure for five minutes and the other was autoclaved for twenty minutes under the same conditions.

For autoclaving, all meals were spread evenly in eighteen

by twelve inch tin trays which were one-half inch deep. After autoclaving, the meals were allowed to cool and were then ground in a Wiley Mill through a one-eighth inch screen. The meals were then vacuum dried at 180° F. for twenty-four hours before analyzing for nitrogen and formulation of the rations.

All rations were isonitrogenous at a protein level of twelve percent and were isocaloric (approximately 1200 Calories metabolizable energy per pound). The same preliminary depletion ration and period used in the previous experiment were used in this experiment. The composition of a typical assay ration is shown on Table IX. These rations differed from those used in the previous experiment in that there was no added corn oil or extract and there was an increase in the amount of cellulose.

In addition to the measures of growth mentioned previously, nitrogen balances were determined on the rations included in this experiment. In this manner it was hoped to gain additional information on the character of the observed growth promotion by obtaining some information as to whether the growth was due to the protein of the meal or to some other component.

Table IX

Composition of Typical Assay Ration

Fed in Experiment Three

Material	Percent of Ration
Ground yellow corn	71.00
Dicalcium phosphate	3.50
Oyster shell flour	0.75
Salt	0.50
Cottonseed meal	14.10
Vitamin, mineral antibiotic premix*	1.00
Alpha Cel	9.15

*Same as in the previous experiment.

Three additional rations were included in the nitrogen balance studies and all three of the rations contained the same unheated hexane extracted meal included above but two of the meals were mixed with enough water to make a slurry and autoclaved for different lengths of time. One sample of meal was autoclaved at atmospheric pressure for five minutes and the other under the same conditions for thirty minutes. After autoclaving, the meals were vacuum dried for twenty-four hours, the nitrogen determined and formulated in rations in the same manner as the other meals of this trial. The chicks used for the additional three nitrogen balances were the same lots of chicks used for testing the

unheated hexane extracted meals.

All balance studies were conducted with groups of four birds for each treatment. The cages were such that they were separated by a four inch space. There were twenty cages and eighteen treatments. Two cages were used as blank controls to determine the amount of feed, feces, and dust which transferred from adjoining cages.

Prior to the collection period of three days, the chicks were placed on the ration which they would be fed during the collection and allowed three days to become acclimated to their new environment. At the end of the acclimation period, porcelain laboratory trays containing one and one-half liters of two percent boric acid were placed beneath the cages, the feed was discarded and fresh feed weighed into each feeder.

An additional weighed amount of feed was added to each trough daily. At the end of the collection period, the feed remaining in the troughs was weighed and subtracted from the gross weight of the feed yielding the net weight of the feed consumed.

At the same time the feed troughs were removed, the collection pans were removed and placed in a forced draft oven at ninety degrees C. for twenty-four hours at which

time the moisture had been removed. The trays and feces were then weighed and representative samples taken for grinding and nitrogen determination. The trays were then thoroughly cleaned, dried and weighed and the amount of material from control cages subtracted. In this manner the total dry weight of the feces was determined.

RESULTS AND DISCUSSION

EXPERIMENT NUMBER THREE

Feeding Trial

Results of the feeding trial with four meals which were autoclaved for different lengths of time are shown on Table X.

The hexane extracted unheated glandless meal was not affected by being autoclaved for two minutes, however, when this meal was autoclaved for five minutes a significant depression in gain and protein consumption resulted. The efficiency with which the protein which was consumed was used was the same for both treatments showing that gain was being affected by autoclaving for five minutes. Autoclaving the meal for fifteen additional minutes did not significantly further decrease its nutritional value.

The hexane extracted heated meal was autoclaved for five minutes before an appreciable effect was observed on its nutritional value. The gain produced by autoclaving the meal for this period of time was about 25 percent below that of the meal which had not been autoclaved. Protein consumption was not significantly affected which gave a protein efficiency much lower than the unautoclaved meal.

As with the previous meal, autoclaving for twenty minutes at 15 pounds pressure did not significantly affect the nutritional value of the meal more than autoclaving for five minutes although there was a tendency for the results to be lower.

Autoclaving the unheated h.a.w. extracted meal for two minutes significantly reduced the gain which it would produce, the protein which the chicks consumed and the efficiency with which the protein was utilized. No additional reduction occurred with five or twenty minutes autoclaving.

Autoclaving the heated h.a.w. meal for two minutes did not affect the gain which it would produce but the protein which was consumed was utilized more efficiently as indicated by the increased protein efficiency. No other changes in the nutritional values of this meal were observed. Although the variation between replications was high for this meal, the results indicate that autoclaving was not as deleterious to this meal as to the others.

The hexane extracted meals from the previous experiment which were included in this trial were either not affected or were drastically reduced in nutritional value by five minutes autoclaving. An additional reduction in their nutritional value was not observed after autoclaving for

Table X

Results of Feeding Trial with

Autoclaved Glandless Cottonseed Meals

Meal & Fraction or Ration	Auto-claving Time (min.)	Gain per Bird day (gms.)	Protein Consumption per Bird day (gms.)	Protein Efficiency Ratio*
Unheated, hexane extracted				
A.	0	3.46±.065	1.33±.030	2.61±.020
B.	2	3.48±.015	1.39±.040	2.48±.085
C.	5	3.15±.149	1.24±.015	2.60±.055
D.	20	3.07±.055	1.30±.090	2.39±.170
Heated, hexane extracted				
E.	0	4.03±.070	1.38±.035	2.99±.045
F.	2	3.98±.005	1.41±.040	2.84±.095
G.	5	3.31±.250	1.35±.140	2.46±.194
H.	20	2.83±.365	1.24±.060	2.27±.193
Unheated, h.a.w. extracted				
I.	0	3.34±.025	1.28±.010	2.61±.005
J.	2	2.99±.034	1.21±.040	2.50±.095
K.	5	2.83±.380	1.17±.040	2.41±.225
L.	20	2.89±.050	1.24±.015	2.35±.005
Heated, h.a.w. extracted				
M.	0	3.53±.410	1.38±.065	2.57±.160
N.	2	3.60±.450	1.26±.075	2.86±.160
O.	5	3.47±.220	1.36±.035	2.55±.085
P.	20	3.18±.160	1.35±.035	2.45±.050
Composite from Trial II				
Q.	5	2.76±.085	1.09±.070	2.56±.145
R.	20	2.75±.020	1.11±.025	2.47±.055

*Gain per gram of Protein consumed.

twenty minutes, suggesting that five minutes autoclaving was sufficient to cause the maximum reduction in nutritional value.

Comparing the unheated and heated hexane extracted meals shows that the heated meals were of uniformly greater nutritional value until the heated meal was autoclaved for five minutes at which time its nutritional value was reduced to that of the unautoclaved unheated meal. From this observation it is concluded that autoclaving of the unheated meal was not the type of heating necessary to increase its nutritional value.

A significant difference between the protein efficiencies of the unheated and heated h.a.w. was not noted as had been observed in previous investigations of these two meals (13). However, the heated meals were consumed in greater quantities and significantly greater gains resulted indicating that palatability may play a major role in the differences observed for meals produced by this solvent system. This observation, which is contrary to that obtained previously from feeding tests with these two meals, might be explained by changes which resulted from the one year storage period which affected their nutritional value.

The heated hexane extracted meal was of the greatest

nutritional value of the meals tested, with the unheated hexane and h.a.w. and heated h.a.w. meals being of equal nutritional value as sources of protein for the chick.

Heating the various fractions in the autoclave at 15 pounds pressure effected changes in both the hexane heated and unheated meals which were different from those observed when the meats were heated for ten minutes at 180° F. and then heated for twenty minutes at 225° F. in the presence of an additional amount of moisture as was done in preparing the hexane extracted heated meal. The results of this experiment and those of the previous experiments show that hexane extracted meals of the greatest nutritive value are produced when the meats rather than the meals are subjected to mild heat treatment in the presence of an added amount of water.

Digestibility Studies

The percent nitrogen utilized from the cottonseed meals fed in this trial and those of the three additional meals fed after completion of the balance trials on Fractions A - R are listed on Table XI.

The percent digestibility was increased 6.14, 18.92 and 3.84 as the time of autoclaving was increased from 0 to 2, 2 to 5 and 5 to 20 minutes, respectively. The overall increase in digestibility was 28.90 percent. The observed increase in digestibility of this meal was not paralleled by an increase in its efficiency as determined by the feeding trial and a decrease in the overall efficiency was noted as the time of autoclaving increased. Therefore, the possibility that the observed increase in growth is due entirely to the protein of the meal is diminished.

The effect of autoclaving on the utilization of the hexane extracted heated meal was not as dramatic as that of the unheated meal and an overall increase of only 2.89 percent was observed. The percent nitrogen utilization of this meal was much higher for the unautoclaved fraction and the two minute autoclaved fraction than for the same fractions of the unheated meals.

Table XI

Nitrogen Utilization of Autoclaved

Glandless Cottonseed Meals

Meal and Fraction or Ration	Autoclaving Time (minutes)	Nitrogen Utilization (percent)
Unheated, hexane extracted		
A.	0	58.13
B.	2	64.27
C.	5	83.19
D.	20	87.03
Heated, hexane extracted		
E.	0	81.47
F.	2	80.47
G.	5	82.94
H.	20	84.36
Unheated, h.a.w. extracted		
I.	0	60.53
J.	2	61.37
K.	5	60.82
L.	20	83.12
Heated, h.a.w. extracted		
M.	0	62.37
N.	2	60.53
O.	5	58.90
P.	20	59.06
Composite from Trial II		
Q.	5	59.09
R.	20	62.59
Unheated, hexane		
S.	0	53.57
T.	5	58.48
U.	30	56.88

After 5 minutes autoclaving, the nitrogen of these two meals was equally well utilized and an additional increase was not found for longer autoclaving times.

The fact that the nutritional value of the glandless cottonseed meals was significantly increased when they were prepared by heating the meats with water before extraction with hexane and that autoclaving decreased the meals' nutritional value but increased the unheated hexane extracted meals' nitrogen utilization strongly indicates two reasons why the meat treatment must be so mild. First, it is necessary that the materials of the meats be changed physically or chemically before maximum nutritional value of the meals can be attained. Secondly, this physical change must be brought about slowly under mild conditions with either solvent or heat so that a particular material or end grouping is not destroyed or altered. These results are in agreement with those obtained by other workers (6, 7, 15, 19) where they found that as the time of autoclaving increased the nutritive value of the meals decreased and the level of free epsilon-amino groups decreased. Since these meals were essentially "free" of gossypol it is hypothesized that the epsilon-amino groups of the glandless meats are either bound by raffinose (20) or are destroyed

when the heating process is too severe.

Utilization of the nitrogen of the unheated h.a.w. extracted meal was not increased after 2 and 5 minutes autoclaving but was increased 22.6 percent when the meal was autoclaved for 20 minutes. The nitrogen utilization of the control ration and that of the meals which were treated for 2 and 5 minutes was approximately twenty percent below that of all of the heated hexane extracted meal whereas growth data for this meal indicate a decrease in its nutritional value as the time of autoclaving is increased.

Autoclaving the heated h.a.w. extracted meal did not affect the chicks ability to utilize its nitrogen while its ability to produce gain was significantly depressed.

These data in addition to the growth data discount the protein of the glandless cottonseed meals as being the only cause of the observed heat and solvent effect.

Utilization of the nitrogen of the three additional rations (Fractions S, T, and U) was improved 5 percent by autoclaving the meal in the presence of enough water to make a slurry. These meals were heated in an autoclave with steam at atmospheric pressure and the dramatic effects due to heat on the nitrogen utilization were not observed.

The thirty minute autoclaving period at atmospheric pressure was either too long or the maximum effect which could be obtained was attained after five minutes autoclaving.

Although feeding tests were not conducted on these meals, from the observed nitrogen utilization data on the same meal (Fraction A) before autoclaving yielded very poor efficiency and nitrogen utilization data. It is therefore doubtful that this treatment resulted in an increase in its ability to produce gain.

MATERIALS AND METHODS

INFRARED SPECTROSCOPY INVESTIGATIONS

To investigate the possibility that the effects observed were due to physical changes induced by heating and extraction with the h.a.w. solvent mixture was the purpose of this experiment. Infrared spectoscopy was the method of choice as a means of studying these phenomena because additional changes in the meals would not be induced and spectral changes might be used to predict their nutritional value.

Although three instruments were used in the course of this study to try to delineate small differences or to obtain greater resolution, the results reported here were obtained entirely on the Perkin-Elmer Model 21 double beam spectrophotometer equipped with a rock salt prism.

All spectra reported were obtained with two 0.05 mm. fixed thickness cells with rock salt windows. The instrument settings were as follows: resolution, 990; response, 1; gain, 6; speed, 3; and a suppression setting of 2.

The four lots of heated and unheated meals used in the previous feeding trial were used as the basis for these investigations because the effect had been observed at

least two times with these lots and both growth and digestibility data had been obtained. Since this investigation was conducted in conjunction with the feeding tests of the previous trial, the same, as well as additional heat treatments were studied.

In addition, thinking that additional information might also be gained on the changes induced by heat in soybean oil meal, three samples of this product were also investigated. These samples were of commercial origin and were prepared in the following manner. The first sample was air dried after having the oil removed by solvent extraction. The second sample was taken during the early stages of desolventization with live steam and the third sample was taken after the flakes had gone through the complete toasting process and was the finished meal.

The meals were prepared for infrared analysis in the following manner. Three replications of the same treatment of each meal were prepared independently and at different times as a means of checking the reproducibility of the results obtained. The sample of meal was taken by using only that fraction which would pass through a sieve with 125 micron openings but would not pass through 105 micron openings. This material was dried by lyophilization with

a Vir-Tis freeze dryer for twenty-four hours. A two gram sample was then weighed into a mortar which contained fifteen mls. of Nujol and mulled with a pestle for about five minutes. The Nujol mulled material was then poured into a sample bottle and sealed. The mull was then usually stored overnight in a refrigerator. After the samples had warmed to room temperature, they were vigorously stirred with a spatula and allowed to stand until only the fine particles remained in suspension in the Nujol. This procedure of sieving and settling was necessary to prevent clogging of the orifice of the fixed thickness cell and to insure a thorough clean out of the cell after each use. To be as quantitative as possible with this technique it was necessary to take the sample to be injected into the cell when the turbidity of each suspension appeared the same. This same procedure was followed throughout the investigation where the mull technique was employed.

The feasibility of obtaining spectra with attenuated total reflectance and attenuated multiple reflectance where little sample preparation and no dispersion media were required, was also investigated. One prism material of silver chloride and one attenuator (Connecticut Instruments Co.) were available. Also, no briquette maker,

which would have decreased the amount of scattered energy, was available at the time of these determinations. Therefore, a fair evaluation of this technique could not be made.

RESULTS AND DISCUSSION

INFRARED INVESTIGATIONS

In conjunction with the feeding tests and nitrogen utilization trials conducted with cottonseed meals the possibility of observing physical changes as they occur in the intact meal was also investigated.

Gorman (10) reviews the evidence from infrared spectroscopy for hydrogen bonding. Huggins (12) discusses the physiological aspects of hydrogen bonding and reviews the work which has been done using infrared and x-ray diffraction as configurational probes in determining the structure of complex organic molecules. Based on present day evidence for hydrogen bonding he theorized that the forces involved might play an important role in the nutritional value of foods.

Sutherland (31) reviews the infrared analysis of the structure of amino acids, polypeptides and proteins and summarizes the wavelengths which are well agreed on as being attributed to configurational changes in the structures of complex organic molecules such as proteins. Some of the hydrogenic stretching frequencies which are well established are:

--- C --- H	between 3.2 and 3.5 microns
--- N --- H	between 2.8 and 2.9 microns
--- O --- H	between 2.7 and 2.85 microns
--- S --- H	between 3.85 and 4.0 microns
--- P --- H	between 4.1 and 4.25 microns.

Some of these frequencies can be modified considerably by hydrogen bonding thus:

--- N --- H	may appear between 3.0 and 3.28 microns
--- O --- H	may appear between 2.9 and 3.4 microns
--- S --- H	may appear between 3.9 and 4.05 microns.

Multiple bond frequencies which might be of concern in this investigation are:

--- C = O	may occur between 5.5 and 6.0 microns
--- C = N	may occur between 5.9 and 6.1 microns
--- C = C	may occur between 6.0 and 6.25 microns

of these only the --- C = O frequency has been observed to appreciably be affected by hydrogen bonding, being

lowered by about 0.2 microns.

Increases in absorption at the wavelengths near 2.75, 3.00 and 6.00 have been observed when a native protein is denatured (1, 29, 32) and are reduced when the protein is renatured but seldom to their original position.

Because the same type of changes may be taking place when soybeans are heated in preparation of the meal this commonly fed protein was also included. The absorption spectra of the cottonseed meals receiving the various treatments are shown in Figures 1 and 2 and the spectra of the three samples of soybean oil meal taken at different times during its commercial manufacture are shown on Figure 3. Both series of spectra were made with unpolarized light. Optical densities are plotted in the ordinate from vertically displaced zero lines marked A, B, C, etc. with wavelength in microns plotted on the axis.

The absorption spectra of the unheated hexane extracted glandless cottonseed meal (Curve A) shows an intense absorption at the --- O --- H, --- N --- H and C = O stretching wavelengths and the --- N --- H deformation wavelength, while the other meals show much weaker absorption in these areas. This is interpreted as meaning that the unheated meal is in a super-folded beta form. This observation is

Figure 1

Infrared Absorption Spectra of Hexane Extracted Cottonseed Meals

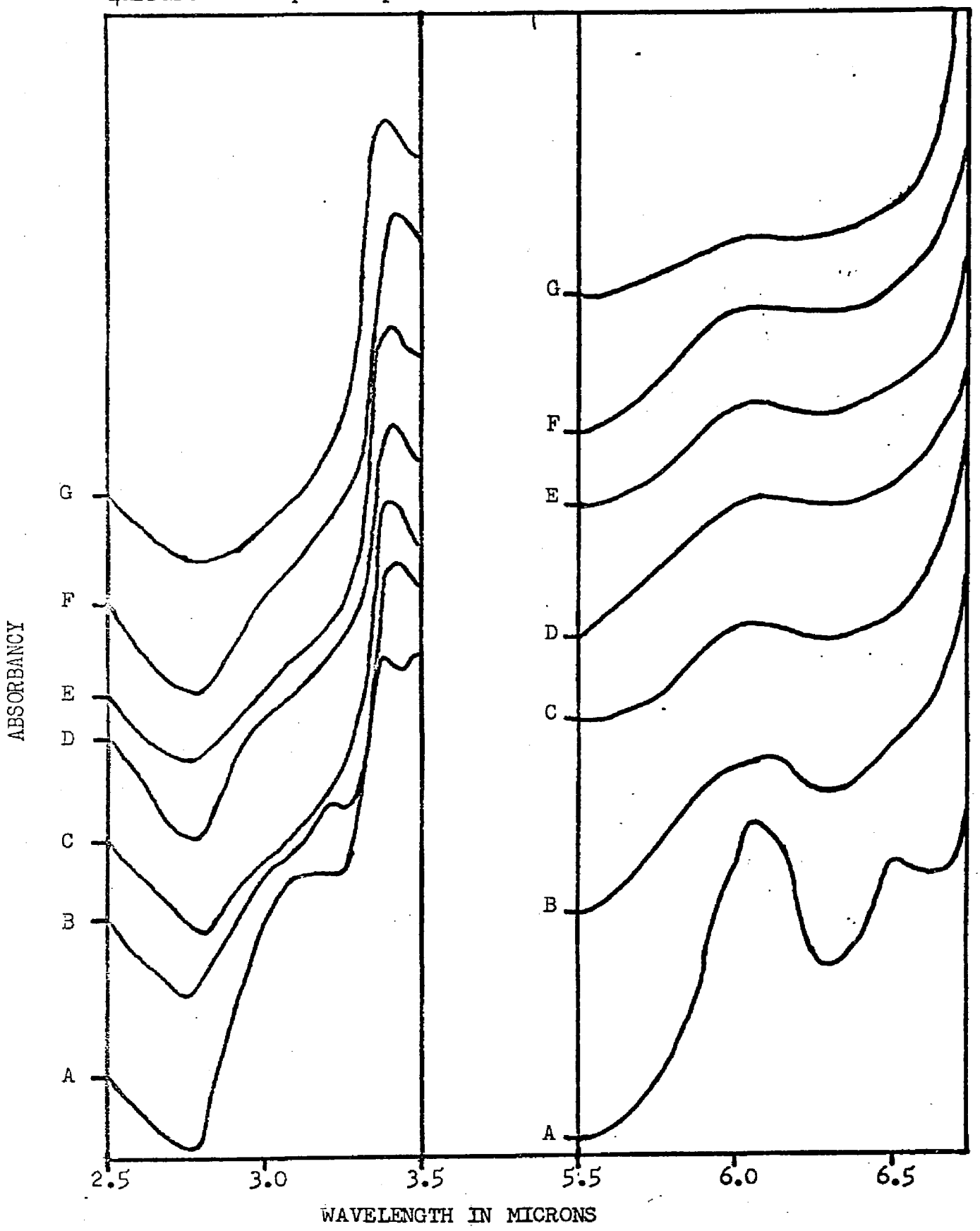

Figure 1 (cont'd)

Spectra A --- Unheated, hexane extracted glandless cottonseed meal.

Spectra B --- Heated, hexane extracted glandless cottonseed meal.

Spectra C --- Unheated, hexane extracted glandless cottonseed meal which was autoclaved for two minutes at fifteen pounds pressure.

Spectra D --- Unheated, hexane extracted glandless cottonseed meal which was autoclaved for five minutes at fifteen pounds pressure.

Spectra E --- Unheated, hexane extracted glandless cottonseed meal which was autoclaved for ten minutes at fifteen pounds pressure.

Spectra F --- Unheated, hexane extracted glandless cottonseed meal which was autoclaved for thirty minutes at fifteen pounds pressure.

Spectra G --- Unheated, hexane extracted glandless cottonseed meal which was autoclaved for forty-five minutes at fifteen pounds pressure.

entirely possible because the unheated hexane extracted meal was desolventized at at least 200° F. for a period of twelve minutes.

The spectra of the heated hexane extracted meal (Curve B) although showing absorption in the same areas as the unheated meal was slightly reduced in intensity and two plateaus are noted instead of one. The plateau at 3.0 microns is believed to be due to --- N --- H which is not hydrogen bonded and that at 3.25 microns is believed to be due to the same grouping but is hydrogen bonded.

From these observations, it would be expected that autoclaving would either fold the material more or would initiate hydrolysis of the protein into single strands or into peptides.

Inspection of Curve C which is the unheated meal which has been autoclaved for two minutes at 15 pounds pressure shows that the amount of energy absorbed in the four major areas of interest is significantly reduced below that of the heated hexane extracted meal after it had been autoclaved for thirty minutes. Curve C shows only one area of significant absorption which is around 6 microns. This area is also reduced to the point where there is almost no absorption due to the C = O stretching

after forty-five minutes autoclaving (Curve G). Autoclaving the heated hexane extracted meal for as short a period as two minutes reduces its absorption spectra to that of the unheated meal which was autoclaved for the same length of time.

The --- N --- H absorption area between 3.00 and 3.25 microns is a different shape for the unheated hexane extracted meal and its counterparts which were autoclaved. It is hypothesized that for the meals to be renatured to produce the highest quality protein it is necessary to heat the meal in the presence of more water over a much longer period of time at lower temperatures than is being done when the meals are autoclaved. This method of preparation appears to be necessary to achieve optimal transfer of hydrogen bonding from the intermolecular form to the intramolecular form.

A third explanation of the changes observed in the absorption spectra could be that on autoclaving the materials which contribute most to the absorption spectra are destroyed or combined in such a manner that they no longer absorb as much energy. This explanation is the most logical based on the feeding trial and nitrogen utilization data where it was noted that as the time of autoclaving increased

Figure 2

Infrared Absorption Spectra of H.A.W. Extracted Cottonseed Meals

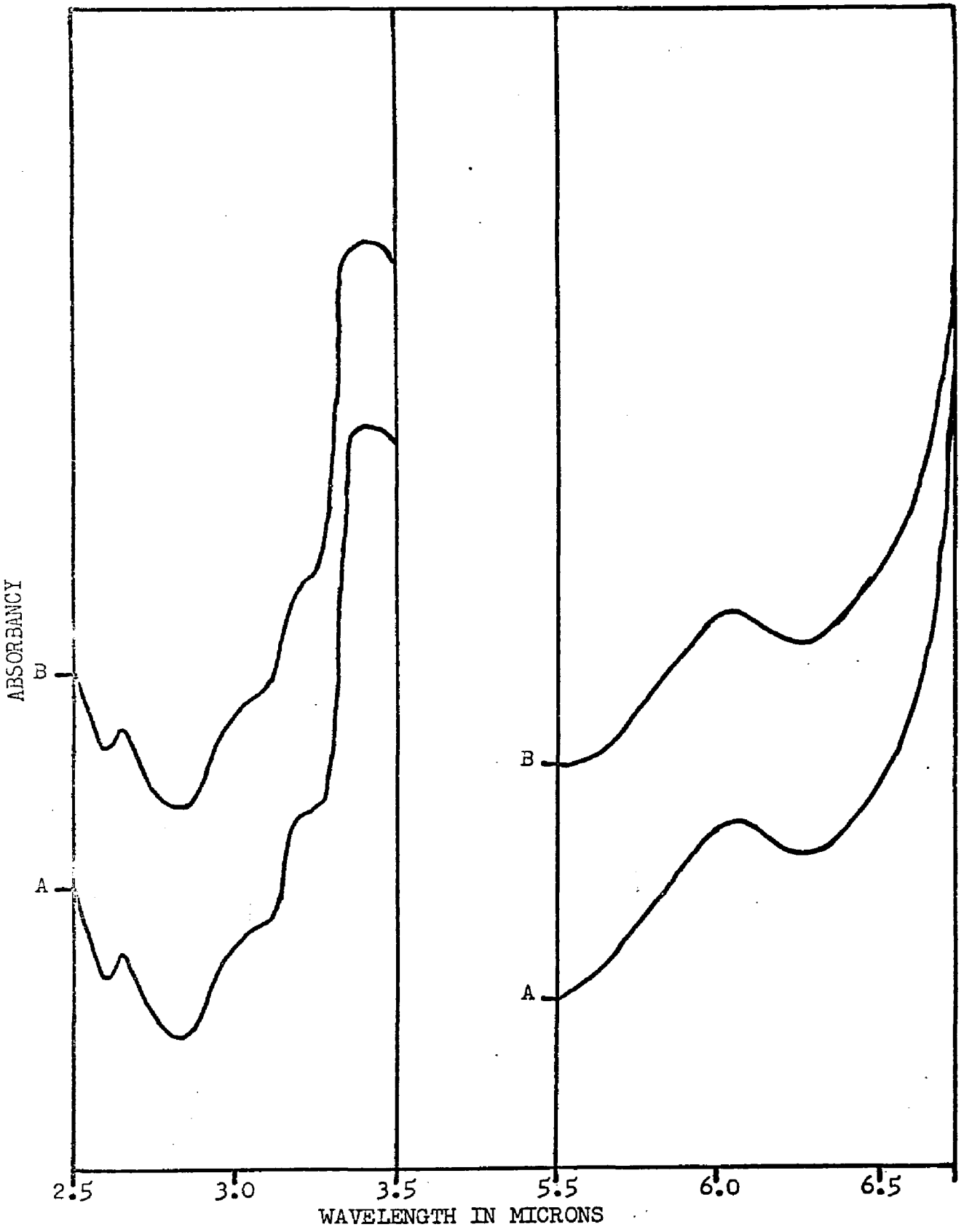

Figure 2 (cont'd)

Spectra A --- Unheated, hexane-acetone-water solvent extracted glandless cottonseed meal.

Spectra B --- Heated, hexane-acetone-water solvent extracted glandless cottonseed meal.

the gain produced decreased whereas the nitrogen utilization increased.

It is concluded from the three investigations conducted in Trial Three that to achieve maximum digestibility and efficiency of utilization of the protein of cottonseed meal it must be heated very gently for about thirty minutes in the presence of an additional amount of water than is in the meal naturally. This procedure is necessary to achieve optimum denaturation of the protein as well as to not destroy or bind certain critical amino acids.

As the time of heating the meal, either over a prolonged period of time in the autoclave or for only twelve minutes, was increased the area of absorption at 6.05 microns decreased in intensity only very slightly with each additional heat treatment. The nutritional value and nitrogen utilization of these meals might be predicted on the basis of these peaks since in this study they appear to be inversely related. With the hexane extracted heated meal being the optimum, as the time of autoclaving increases (Curves C through G) the intensity of this peak diminishes gradually with each additional autoclaving period. The exception to this hypothesis is the unheated hexane extracted meal which absorbs much more strongly in this area but yields

the poorest growth and nitrogen utilization data. It therefore appears that on either side of the optimum there is a decrease in the nutritional value of the meal. The areas of absorption from 2.75 to 3.50 microns are of consequence when predicting the gross nutritional value of the meal but are inconsequential when finer degrees of value are to be considered.

Inspection of the spectra of the glandless meals produced by extracting the meats with the h.a.w. solvent system (Figure 2) both with and without heat show very little difference between the two spectra, which is to be expected from their growth and nitrogen utilization data. However, by comparing these spectra with those of the hexane extracted meals, it is apparent that the curve of the heated hexane extracted meal is similar with the h.a.w. curves. The h.a.w. extracted meals show less intense absorption at the --- O --- H stretching wavelength and more intense absorption at the --- N --- H stretching wavelength both where the group is hydrogen bonded and not hydrogen bonded. The --- C = O stretching frequency is about the same for the meals prepared by the two solvent systems with slightly more intensity for the h.a.w. extracted meals. A rigid interpretation of the differences of the

meals prepared with the two solvent systems cannot be made because the sample thickness of the two meals was different when their spectra were run.

The spectra obtained for the soybean oil meals show slight increases in absorption as the meal goes from one stage of processing to another. The increases in intensity of the absorption spectra at 2.75, 3.25, 6.10 and 6.50 microns indicates that the protein is denatured as the heating process proceeds. However, until additional investigations have been made, it is debatable if a great deal of significance can be attached to these small changes in their absorption spectra.

Figure 3

Infrared Absorption Spectra of Soybean Oil Meals

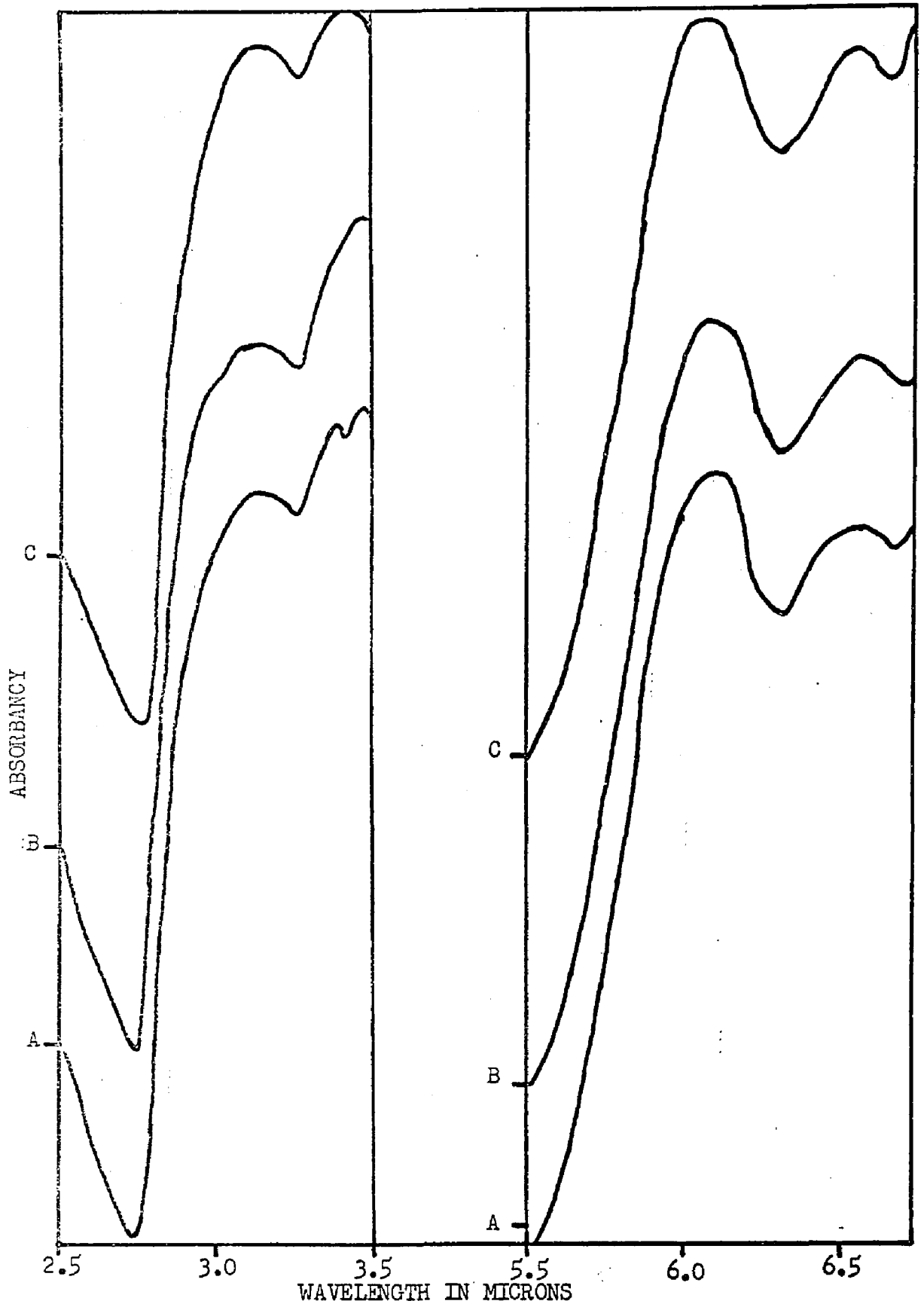

Figure 3 (cont'd)

Spectra A --- Air dried flakes after having the oil removed by solvent extraction.

Spectra B --- Flakes from the early stages of desolventization with live steam.

Spectra C --- Flakes after complete desolventization with live steam and the toasting process is complete.

SUMMARY

Glandless cottonseed meals have been produced at this laboratory which duplicate those which were produced at the Texas Agricultural and Mechanical College and the Southern Regional Research Laboratory. Extracting the glandless meats with a hexane-acetone-water solvent system produced meals with the highest nutritive value while meals which were extracted with hexane and subjected to mild heat treatment over a prolonged period of time were slightly lower in nutritional value. A meal which was extracted with hexane and not heated was the poorest in nutritional value. In another series of extractions, the heated hexane extracted meal was of greater nutritional value than the h.a.w. extracted meal.

The recombination of either the heated or unheated h.a.w. extracts or the unheated hexane extract with the h.a.w. extracted meal produced the same magnitude of growth depression. Additional extraction of the hexane extracted meal with the h.a.w. solvent mixture produced a meal which was equal in nutritional value to the meal which was prepared by extracting the meats with the h.a.w. solvent alone. The addition of the small amount of h.a.w. extract

removed to the above meal which had been extracted with the two solvent systems tended to produce the greatest growth and protein consumption of all the meals of this test. Additional extractions were made and the unheated and heated extracts from each extraction were tested in combination with soybean oil meal rather than the cottonseed meals from which the extracts were obtained. Results of this test demonstrated that all unheated extracts and unheated corn oil produced equal growth. Heating the extract from the unheated hexane extracted glandless meal produced a significant increase in growth and protein consumption. The other extracts which included extracts from the h.a.w. extracted meal, an extract from the meal prepared by extracting the meats with hexane without heat, an extract from meals prepared by heating the meats very mildly in the presence of an additional amount of water were significantly reduced in nutritional value when they were heated. The same reduction was observed with heating corn oil.

The fact the extracts which should have contained the factor and those which should have been devoid of the factor responded in the same manner on heat treatment appears to eliminate the possibility that the deleterious factor is

removed in the extraction process. It is possible, however, that the principle responsible for the reduction in nutritive value is destroyed in the process of extraction.

The reason for the depression in the gains produced by the heated extracts and heated corn oil is hypothesized to be due to the production of free radicals or the chick edema factor both of which are toxic to the chick.

Extraction of the glandless meats with hexane after they had been heated in the presence of 12 percent water for thirty minutes produced significantly greater gains than when the meal was extracted with hexane without heating or the added water, but the gains of the heated meal could be reduced to those of the unheated meal by grinding the meal to an extremely fine particle size and could be further reduced by drying the meal under vacuum with heat for a period of 24 hours. These results plus the additional gains produced when the meal was first extracted with hexane without heat and then extracted with the h.a.w. solvent system strongly suggest that the observed effect is due to a physical or chemical change in the complex structure of the meal which requires the presence of water and mild heat or water and acetone before the reaction can take place. This observation was further substantiated

when a meal was produced by heating the meats to 180° F. for ten minutes and then adding 12 percent boiling water and heating the meats for an additional 20 minutes which promoted greater gains, protein consumption and protein efficiency values than any other cottonseed meal produced and was equal to soybean oil meal as a source of protein for the chick.

In attempting to increase the nutritional value of the unheated hexane extracted meal to that of the heated hexane extracted meal it was found that autoclaving the unheated meal for as little as two minutes at 15 pounds pressure lowered its nutritional value. Additional times of autoclaving also decreased the nutritional value of the meal but increased its nitrogen utilization significantly. Autoclaving the heating hexane extracted meal for as long as twenty minutes did not appreciably change its nutritional value.

The unheated h.a.w. extracted meal was significantly reduced in nutritional value after it was autoclaved for two minutes but the chicks' ability to utilize its nitrogen was not altered. The heated h.a.w. extracted meal was reduced in nutritional value only after twenty minutes autoclaving at 15 pounds pressure but its nitrogen utilization was not altered. These data show that heating by

autoclaving is not the method of choice to produce the highest quality protein from glandless cottonseed. They further suggest that the reason for the decrease in nutritional value of the meal as the time of autoclaving is increased is due to some material being combined with the meal or a particular component in the meal is destroyed which results in an increase in the nitrogen utilization but lowers its nutritional value. The hypothesis of choice to explain these findings appears to be that certain critical amino acids are removed or are combined in the process of autoclaving.

Infrared spectroscopial investigations of the heated hexane extracted meal, the unheated hexane extracted meal and its fractions which were autoclaved for varying lenghts of time, and the unheated and heated h.a.w. extracted meals were made. The results of these investigations reveal that the area of absorption from 2.75 to 3.25 microns is very important when attempting to predict the gross nutritional value of the meal but of little use when trying to predict nutritional differences of lesser magnitude. The area of absorption at 6.05 microns appears to be related to those nutritional differences which are smaller in magnitude.

Both the heated and unheated h.a.w. extracted meals

and the heated hexane extracted meal showed a significant absorption at the wavelengths for the --- N --- H which was hydrogen bonded. These are the only meals which showed this absorption characteristic but it may be masked by the broad spectrum shown for the unheated hexane extracted meal. This observation and the results of the feeding tests and nitrogen balances indicate that for optimal nutritional value to be attained the proper balance between the --- N --- H which is hydrogen bonded and that which is not and the number of carboxyl carbonyl groups must be attained.

Absorption of soybean oil meal determined under the same conditions as the cottonseed meal reveals no significant differences in its spectra as the processing time increases but suggests the need for a more thorough investigation of its absorption characteristics.

CONCLUSIONS

The results obtained from this study indicate the following conclusions may be drawn:

(1) High quality glandless cottonseed meals can be produced on a small scale in the laboratory.

(2) The proportion of hexane to acetone when 2 percent water (volume for volume) is used must be 33.5 and 64.5, respectively to produce a homogeneous solvent mixture.

(3) The deleterious property of hexane extracted glandless cottonseed meal is not present in the h.a.w. extract nor is it present in the hexane extract after the meats are heated mildly over a prolonged period of time.

(4) Corn oil, the hexane extract from seed which have been heated and the h.a.w. extract are significantly reduced in nutritional value when they are heated for thirty minutes at a temperature of 225° F.

(5) Glandless cottonseed meals of the highest quality are produced by heating the meats to 180° F., adding 12 percent boiling water in the form of a spray and heating the meats at 225° F. for an

additional period of 25 minutes before extraction with hexane.

(6) The beneficial effect of extracting the heated meats with hexane is significantly reduced when the meal is ground to a very fine particle size and vacuum dried for twenty-four hours.

(7) Autoclaving at 15 pounds pressure for two minutes is a much too severe heat treatment to increase the nutritional value of the unheated hexane extracted meal and decreases in the nutritional value of the meal are noted after five minutes autoclaving.

(8) Autoclaving the unheated and heated hexane extracted and the unheated h.a.w. extracted meal for a period of five or twenty minutes significantly reduced their nutritional value to the chick as measured by gain per chick day and protein consumption per chick day.

(9) As the time of autoclaving the unheated hexane extracted meal was increased from two to twenty minutes the nitrogen utilization of the meal was increased 29 percent and the gains were decreased 11 percent.

(10) Nitrogen utilization of the unautoclaved heated hexane extracted meal was significantly higher than the h.a.w. extracted meals and the unautoclaved unheated hexane extracted meal.

(11) Autoclaving the cottonseed meals does not produce the same type of a change as does heating the meats or meal over a prolonged period of time at lower temperatures.

(12) Evidence from infrared investigations indicate that the same type of a change is induced by mild heat that is induced by extracting the meats with the h.a.w. solvent system and that these changes are physical in nature and associated with the amino and carboxyl carbonyl structure groups.

(13) The improvement in the nutritive value of the unheated hexane extracted glandless cottonseed meal appears to be due to changes which involve the peptide linkages of the protein and the extent of hydrogen bonding between these linkages.

SELECTED BIBLIOGRAPHY

1. Ambrose, E.J., 1951.
   Infra-red spectra and structure of fibrous proteins. Proc. Roy. Soc. (London) A206: 206.

2. Cabell, C.A. and I.P. Earle, 1956.
   Relation of amount of protein and quality of protein in the diet to free gossypol tolerance by the rat. J. Am. Oil Chemists Soc. 33: 416-419.

3. Carruth, F.E., 1917.
   Methods of approximating the relative toxicity of cottonseed products. J. Biol. Chem. 32: 87-90.

4. Clark, E.P., 1928a.
   Studies on Gossypol. II. Concerning the nature of Carruth's gossypol. J. Biol. Chem. 76: 229-235.

5. Clark, E.P., 1928b.
   The composition and toxic effects of gossypol. Oil and Fat Ind. 5: 237-242.

6. Condon, M.Z., E.A. Jensen, A.B. Watts and C.W. Pope, 1954.
   Effect of autoclaving in the presence and absence of gossypol. J. Agr. and Food Chem. 2: 822-826.

7. Conkerton, E.J., W.A. Martinez, G.E. Mann and V.L. Frampton, 1956.
   Changes induced by autoclaving a solvent-extracted cottonseed meal. J. Agr. and Food Chem. 5: 460-463.

8. Fulton, H.J., 1938.
   Hopi cotton, a variable species. J. Agr. Res. 56: 333-342.

9. Gallup, W.D., 1927.
   Heat and moisture as factors in the destruction of gossypol in cottonseed products. Ind. and Eng. Chem. 19: 726-728.

10. Gorman, M., 1957.
The evidence from infrared spectroscopy for hydrogen bonding. J. Chem. Educ. 34: 304-306.

11. Grau, C.R., and P. Zweigert, 1955.
The effects of various processing methods on the value of cottonseed meal as an amino acid source for chickens. J. Poul. Sci. 34: 724-728.

12. Huggins, M.L., 1962.
Physiochemical aspects of hydrogen bonding and their application to biology. Amer. Sci. 50: 485-496.

13. Johnston, C., 1961.
Nutritional studies with glanded and glandless cottonseed meals. A Masters Thesis, Louisiana State University.

14. King, W.H., J.C. Kuck, and V.L. Frampton, 1961.
Properties of cottonseed meals prepared with acetone-petroleum ether-water azeotrope. J. Am. Oil Chemists Soc. 38: 19-21.

15. Kuiken, K.A., 1952.
Availability of the essential amino acids in cottonseed meal. J. Nutr. 46: 13-25.

16. Lewton, F.L., 1912.
The cotton of the Hopi Indians: a new species of Gossypium. Smithsonian Misc. Coll. 60: 1-10.

17. Lyman, C.M., B.R. Holland and F. Hale, 1944.
Processing cottonseed meal. A manufacturing method for eliminating toxic qualities. Ind. and Eng. Chem. 36: 188-190.

18. Mann, G.E., F.L. Carter, V.L. Frampton, A.B. Watts and C. Johnston, 1962.
Evaluation of cottonseed meals prepared by extraction with acetone-hexane-water mixtures. J. Am. Oil Chemists Soc. 39: 86-90.

19. Martinez, W.H. and V.L. Frampton, 1958.
Lysine content of cottonseed meals. J. Agr. and Food Chem. 6: 312.

20. Martinez, W.H., V.L. Frampton and C.A. Cabell, 1960.
Effects of gossypol and raffinese on lysine content and nutritive quality of proteins in meals from glandless cottonseed. J. Agr. and Food. Chem. 9: 64-66.

21. Mattson, J.B., R.A. Martin and R.A. Volpenhein, 1960.
The gossypol content and oil composition of "gossypol free" cottonseeds. J. Am. Oil Chemists Soc. 37: 154.

22. McMichael, S., 1959.
Hopi cotton, a source of cottonseed free of gossypol pigments. Agronomy J. 51: 630.

23. Miles, J.E., 1963.
Nutritional studies with chelated minerals.
A Masters Thesis, Louisiana State University.

24. Milligan, J.L. and H.R. Bird, 1950.
Effect of processing variants on the nutritional value of cottonseed for chicks. J. Poul. Sci. 30: 651-657.

25. Olcott, H.S. and T.D. Fontaine, 1941.
The effect of autoclaving on the nutritive value of the proteins in cottonseed meal. J. Nutr. 22: 431-437.

26. Olcott, H.S. and T.D. Fontaine, 1942.
Effect of cooking on solubility of cottonseed meal proteins. Ind. and Eng. Chem. 34: 714-716.

27. Osborne, T.B. and L.B. Mendel, 1917.
The use of cottonseed as food. J. Biol. Chem. 29: 289.

28. Patrick, H., 1953.
The effect of processing method and certain supplements on a lysine deficiency in cottonseed meal for chicks. J. Poul. Sci. 32: 942-944.

29. Rudall, K.M., 1952.
The proteins of the mammalian epidermis. Advances in Protein Chemistry 7: 253.

30. Smart, F.H., C.L. Rhyne and V.W. Smart, 1960.
Dietary evaluation of cottonseed protein from cotton bred for low gossypol content. J. Agr. and Food Chem. 9: 82-84.

31. Sutherland, G.B.B.M., 1952.
Infrared analysis of the structure of amino acids, polypeptides and proteins. Advances in Protein Chemistry 7: 291.

32. Uzman, L.L. and E.R. Blout, 1950.
Infra-red spectra of films of native and denatured pepsin. Nature 166: 862.

33. Wamble, A.C., 1963.
Personal communication.

34. Withers, W.A. and F.E. Carruth, 1915.
Gossypol, the toxic substance of cottonseed meal. J. Agr. Res. 5: 261-268.

AUTOBIOGRAPHY

I was born in Siloam Springs, Arkansas on March 4, 1937, the third son of Dick D. and Juanita Johnston. When I was five years of age we moved to Fayetteville, Arkansas, where we lived for two years and I began grammar school. We then moved to Jamuahl, California, where we lived for a year and a half, and I completed the second grade. We then moved back to Fayetteville, where my parents now live.

I completed grammar school in Fayetteville and was graduated from Fayetteville High School in June of 1956. The following September I entered the University of Arkansas majoring in Poultry Nutrition.

On January 24, 1958, I married the former Sybil Joyce Chaffin of El Dorado. On August 3, 1959, our first child, Anne Elizabeth was born.

In January, 1960, I was granted the degree of Bachelor of Science from the University of Arkansas.

In February, 1960, I entered Graduate School at Louisiana State University, being employed as a Graduate Assistant in the Department of Poultry Industry. I was granted the degree of Master of Science in June, 1961. At that time I accepted the position of Associate in

the Department of Poultry Science and began working toward the degree of Doctor of Philosophy, majoring in Poultry Nutrition and minoring in Biochemistry.

On April 16, 1963, our second child, Mary Jane, was born.

EXAMINATION AND THESIS REPORT

Candidate: Charles Johnston

Major Field: Poultry Nutrition

Title of Thesis: The Characterization of a Growth Inhibitor of Glandless Cottonseed

Approved:

A B Watts
Major Professor and Chairman

Mrs Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

Arthur F. Novak

Ernest A. Epps Jr.

William A. Johnson

Jordan G. Lee

Date of Examination:

July 26, 1963